# STUDIES ON CHEMICAL CHANGES DURING THE LIFE CYCLE OF THE TENT CATERPILLAR (MALACOSOMA AMERICANA FAB.). I. MOISTURE AND FAT[1]

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Aside from studies on the chemical composition of the silk worm, information regarding changes taking place during the life cycle of insects is extremely rare. There are a number of reports published on the moisture content of insects during pupation, some on changes of weight during this period (gross determination of changes), a few isolated results are available on chemical composition of larvæ, pupæ and eggs, but more detailed analyses made during the complete life cycle are wanted. The information on hand concerned with the chemical composition of the silk worm, made in an effort to gather data for the better understanding and propagation of this economically important insect, deals practically entirely with the larvæ and their food plants, and some with the cocoons. Some work has been done on the $CO_2$ output of insects but this registers activity of the insect and not definite accumulation and decomposition of all materials.

The importance of chemical information is obvious if we wish to interpret biological activities in concrete terms. If sufficient analyses of different insects are amassed, our fundamental knowledge of the biochemical changes will throw light upon problems in ecology and insect control, give new aspects and impetus, and doubtless render important results. The relation between biochemical activities and geochemical factors are especially interesting. The relation between insect and food plants, geographical distribution of insects and composition of soils, which in turn affects food plants, the effect of climate on insect distribution and activities will all need in the end correlation with biochem-

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ical changes taking place in the insects. From an economical point of view the most important problem for the near future is securing more knowledge about the physiology of insects. If we have a better understanding of the changes taking place in the eggs, larvæ, pupæ and adults control measures will present themselves which are now overlooked or discarded as inadequate. In this relation Swingle's (3) paper on digestive enzymes of an insect is especially interesting. Fortunately we have a mass of material published on the structure of insects, which will be of a tremendous aid in interpretation and application. Comparative anatomy and classification will be guides and help, since without this ground work chemical data obtained would necessarily be of limited importance and use.

### *Methods and material*

About one thousand egg masses of the tent caterpillar were collected[1] in 1924, kept in an insectary and lots of 50–70 egg masses used for analyses. The caterpillars upon hatching were analyzed in lots of 50–1000, depending upon the size, and pupæ and adults again were analyzed in groups of about 50 individuals. At the outset individual egg masses were used and also some analyses were made of individual larvæ, pupæ and adults.

The material was analyzed for moisture content, total ash, insoluble ash, ether extract[2] (fats), total nitrogen and sulfates, while analyses of amino acids, ammonia, albumoid nitrogen, sugars, phosphorus, carbonates and chlorine were made occasionally. Official methods were employed unless otherwise noted. Weight, length, and width of the heads of adults were obtained. To study possible effects of atmospheric conditions batches of egg masses were submitted for 66 hours to a constant temperature of 80° F. and constant atmospheric moisture content of 73.4 per cent. with lots taken directly from the insectary analyzed at the same time.

### *Results*

The results given in this paper are a part of the data secured. Other results will be published at an early date. The results reported here are on lots as stated above.

[1] Thanks are due Mr. Carl Ilg, assistant in the Entomology Department, for the collection of all the material used.

[2] Terms fat, fatty substances, etc., refer to ether extract.

The egg masses contain on an average from 300 to 400 eggs. It is extremely difficult to remove the gelatinous cover from the eggs. At first the cover was removed as far as possible by hand, analyzed, compared with the analyses of the formed larvæ taken out of the egg cases and with the analyses of the whole mass. Later on a method was found to dissolve the gelatinous cover without apparent attack on the larvæ in the egg cases. It was found that the cover has practically no ash content and consist mainly of nitrogenous material. Microscopic examination of the larvæ from egg masses treated to dissolve the gelatinous covers and egg cases showed that the tiny larvæ were non-transparent, indicating that they were not harmed nor their gut contents removed by the treatment. However the results presented in the graphs are based on the whole egg masses. The following figure (1) represents the per cent. moisture and per cent. of ether soluble material during the different stages in the life cycle of the insect.

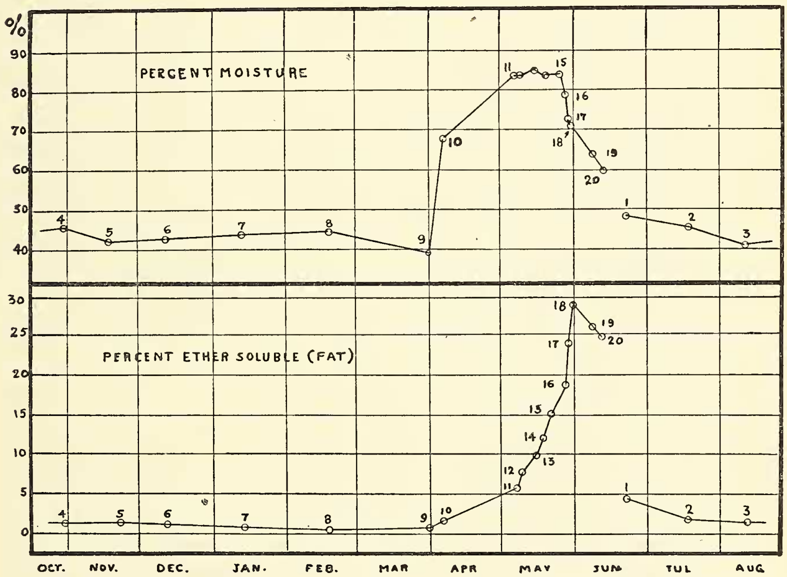


FIG. 1. Per cent. moisture and per cent. fats present during the life cycle of tent caterpillars. 1 = eggs in liquid stage; 2 = larvae nearly all formed; 3–8 = eggs; 9 = newly hatched larvæ; 10–15 = growing lavæ; 16 = ready for pupation; 17 = prepupal stage; 18 = larvæ just pupated; 19 = ready for emergence; 20 = adults.

The moisture content of the egg masses shortly after deposition was 48.5 per cent. The well formed larvæ remain approximately 9 months in the egg cases and during this time the moisture fluctuated somewhat until upon hatching the young caterpillars contained 39.4 per cent. moisture. The first 6 days after hatching the moisture content increased to 68.5 per cent., while by the time the caterpillar larvæ had an average weight of 50 mgr. (end of second instar) the moisture content had reached 85.1 per cent., it remained there with minor fluctuations until the larvæ were full grown (average weight 685 mgr.). As might be expected when the larvæ made ready for pupation (becoming flabby and shrunken) the moisture content went down. After pupation the per cent. moisture was 71 and the adults (26 males and 22 females) contained still 60 per cent. moisture.

Ether soluble materials (fats) of the egg masses (eggs in the liquid stage) was 4.45 per cent., decreasing very rapidly during the first few weeks, until at the time of hatching only 0.56 per cent. was left. Since the tiny caterpillars constitute about 48 per cent. of the total mass, the total fat present in the newly laid eggs was about 9 per cent. In spite of the fact that the young caterpillars needed large quantities of food for their growth activities they were able to store up some fatty materials. From the time when they weighed but 20 mgr. (second instar; No. 11 on the curves) until they were full grown (No. 16) fat increased very rapidly. All figures are based on the dry weight of the caterpillars including the gut contents. Full grown caterpillars kept in a cage ejected in 40 hours a little less than one fourth of their total wet weight. The droppings contained 1.06 per cent. ether soluble material of a very dark green color (chlorophyll). Calculated on this basis the full grown larvæ contained (before they were ready for pupation) about 19 per cent. fat while the flabby and shrunken larvæ analyzed 18.71 per cent. fat; this checks very well and numbers 10 to 16 contained actually one-fourth more fat than is shown in the graph. During the prepupal stage (larvæ in semi-liquid form taken out of their cocoons) fat content appeared to increase but at the same time moisture content decreased. After pupation the fat content appeared to decrease from 28.82 per cent. to 24.72 per cent. in the adults.

One would expect that the fat and moisture content of the egg masses would decrease with the formation of the larvæ; also that fat would decrease after the larvæ stopped taking food. However, this was not the case. Keeping in mind that the fat content is based on the dry weight of the material we see that fat increased rapidly during the prepupal period. From the time no food was taken (no. 16) until the larvæ had just pupated (no. 18) the actual fat content increased with 67 per cent. of the fat content present at the first (no. 16) stage. It seems clear therefore that the organism while in a transition stage, at a time when one would presume that all cells are engaged in the formation of substances needed for metamorphosus, certain parts are still continuing the production of fatty substances. The amount of fatty material formed in this period is less than half used up during the chrysalis stage.

Snodgrass (2) says that "the fat bodies separate from one another in the pupa and float about in the body cavity as a mass of globular cells. They give up their oil droplets either by absorption or by the dissolving of their thin walls which scatter their contents broadcast in the blood."

From our results it would seem that when the oil droplets are set free and apparently visibly disappear or scattered in the blood of the insects they may in reality be emulsified by substances in the blood and possibly partly hydrolyzed later on. The reason for this assumption is that the fatty substances present in the chrysalis are only partly used while an appreciable amount seems to be retained for energy and for the formation of the eggs. It might be stated here that during the time of metamorphosis a remarkable fluctuation of the nitrogen-fat ratio occurs. About 10 per cent. of the total fat present disappeared from the time the larvæ had just pupated until the adults emerged. This 10 per cent. seems to be used in the processes of reconstruction. The balance of the fatty material in the female was used in part for the formation of the eggs, some for energy supply of the adult and some deposited in the eggs. Snodgrass states that the male moths upon emergence contain an abundance of fat tissue, the cells of which are filled with droplets of fatty oil, while the body of the female contains no fat tissue but her ovaries are full of mature eggs ready to be laid. Our figures show that the body of the adult contains an average of 19 mgr. of fatty substances, while the newly laid egg masses contained 3 mgr. on an average. It seems then that the female adult had still a quantity of fatty material in its body although it could not be detected by miscroscopic examination.

The amount of moisture in the egg masses varying from 42 to 48 per cent. seems comparatively low. Tichomiroff (4) found from 64.4 to 65.8 per cent. moisture in the eggs of *Bombyx Mori.* Our low results are caused by the fact that moisture is here calculated on the basis of egg masses. Larvæ taken out of the egg cases in November contained 61.9 per cent. moisture and 60.2 in January. The moisture content of newly hatched caterpillar larvæ was about 40 per cent., while Kellner (1) reports about 76 per cent. for newly hatched silk worms. Moisture in growing caterpillars fluctuated between 82 and 86 per cent. This agrees with the findings of Kellner for larvæ of the silk worm containing from 83 to 88 per cent. moisture.

The rapid increase of fats in growing caterpillars was not observed by Vaney and Maignon (5) in the silk worm. They found a progressive decrease of fat but a rapid increase in glycogen reaching its maximum when the larvæ were full grown and decreasing continuously during pupation. They concluded that the pupæ used fats for their metamorphoses. It can be seen from our figures that total fats calculated on a dry basis increased persistently in the larvæ and during the first stages of metamorphosis until the pupæ were well formed (fig. 1). From then on reduction took place and the decrease of fats kept pace with the decrease of moisture.

The relation between fat and moisture graphically shown in fig. 2 is of special interest. Calculating the fat contents on the basis of the same moisture throughout the life cycle, the curve shows a rapid decrease of fats in the eggs until the larvæ are well formed and from then on practically no decrease until January, showing that only extremely small quantities of fat are necessary for maintenance of the larvæ while confined in the egg cases. A more rapid decrease occurred apparently when hatching was approaching. The slight rise just before hatching

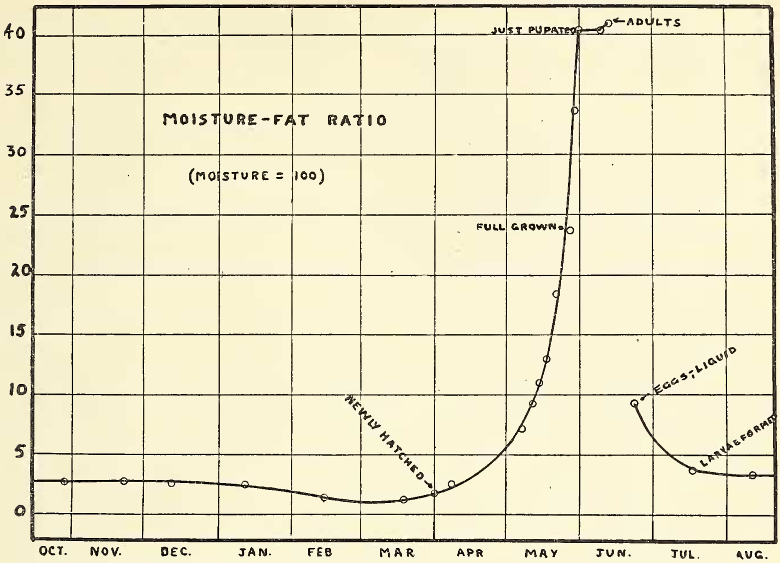


FIG. 2. Relation between moisture and fats during different stages of the life cycle of tent caterpillars. Fat calculated to a constant moisture basis of 100.

is due to calculating fat on the whole eggmass for the months June–March. The rapid increase of fat during May terminating at the time when the larvæ had just pupated shows that the conclusions drawn from figure 1 are correct. Since moisture decreased more rapidly during the period between "full grown" and "just pupated" the results for fat are accentuated. During the pupal stage moisture and fat decreased at a same rate resulting in a flattening of the curve, while in the last stage moisture again decreased more rapidly than fatty substances.

The amount of fat present in the newly deposited eggs (without egg cases or cover) was about 9 per cent. This quantity diminished rapidly during the first weeks, indicating that metabolic activities during embryonic development are great. Consequently if control measures were to be used against the eggs these should be employed at this time rather than at the time when the larvæ in the eggmasses are comparatively inactive. This might hold as well for other insects. Metabolic processes do not seem as great in the pupal stage and it would presumably be more difficult to kill the insect at this stage than at any other, even if substances are found which will penetrate the hard skin of the pupæ.

*Summary*

Chemical analyses made on the tent caterpillar during its life cycle are reported in part.

Moisture content decreased gradually in the egg masses, increased rapidly in the young growing caterpillar (first two instars), remained constant until they were fullgrown, and decreased again rapidly from the time they were ready for pupation until the adults emerged.

Moisture was lowest at the time of hatching (39.4 per cent.) and highest during third to fifth instars, namely 83–85 per cent.

Ether soluble material (fats) decreased fairly rapidly in the eggs after deposition, decreased slowly until the larvæ hatched, increased gradually during the first two instars, increased rapidly during the next three instars, and increased at an accelerated rate during the first part of metamorphoses, while it decreased during the second part.

Fat calculated on a dry basis was lowest upon hatching (0.66 per cent.) and highest when larvæ had just pupated (28.8 per cent.).

The relation between moisture and fatty substances during the life cycle is graphically shown.

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